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Date:	April 19, 2006	**Pages:**	32 (including cover sheet)		
Re:	Application No. 09/627,796 Date Filed: July 28, 2000 Confirmation No. 3581 Title: Non-Nucleic Acid Probes, Probe Sets, Method and Kits Pertaining To The Detection Of Human Chromosomes X, Y, 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 16, 17, 18 And 20 As Well As 13/21 As A Pair Our Ref No. BP9806US-CP2				

Attached is a Request for Final Agency Decision.

Please send confirmation of receipt to fax no. 508-383-7468.

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Thank you.

Pat Tocci
IP Paralegal

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BP9806US-CP2

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Serial No:	**09/627,796** **Confirmation No. 3581**
Date Filed:	July 28, 2000
Application Title:	Non-Nucleic Acid Probes, Probe Sets, Method and Kits Pertaining To The Detection Of Human Chromosomes X, Y, 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 16, 17, 18 And 20 As Well As 13/21 As A Pair
Applicant:	Krishan L. Taneja
Group Art Unit:	1634
Examiner:	Jehanne Souaya Sitton
Action Type:	Reply To The "Decision On Petition" dated **February 27, 2006**

Certificate of Transmission:
37 C.F.R. § 1.8

I hereby certify that this correspondence is being facsimile transmitted to the U.S. Patent and Trademark Office (Fax No. 703-872-9306) on this 19th day of April, 2006.

Brian D. Gildea
Reg. No. 39,995

Attention: Deputy Commissioner For Patent Examination Policy:

Petition to the Deputy Commissioner For Patent Examination Policy
under 37 C.F.R. § 1.181 and M.P.E.P. § 1002.02(b)
For Review of the "Decision On Petition" dated February 27, 2006

- REQUEST FOR FINAL AGENCY DECISION -

Deputy Commissioner for Patents
United States Patent & Trademark Office
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir or Madam:

This petition is filed for review of the "Decision on Petition" issued by Jasemine Chambers, Director, Technology Center 1600 on February 27, 2006. No fee is considered necessary for this petition, but the Office is hereby authorized to deduct any appropriate

fee due for the consideration of this Petition to Deposit Account No. 01-2213 (Order No. BP9806US-CP2). Accordingly, please consider the following petition.

I. Preliminary Statement

On October 25, 2005, a petition under 37 C.F.R. § 1.181 and M.P.E.P. § 1002.02(b) requesting review of the "Decision of Petition" dated August 30, 2005 by the Deputy Commissioner For Patent Examination Policy was requested.[1] Simply stated, this was a request for a final agency action. In response to this request, the "Decision on Petition" dated February 27, 2006 was received – which is not a final agency action since it was signed by a Technology Center Director and not by the Deputy Commissioner for Patent Examination Policy. For the avoidance of any doubt whatsoever, with this petition Applicant seeks a final agency decision that can be appealed to District Court if adverse.

II. Status of the Application

On October 17, 2005, the Office issued an action stating:

> *This application is in condition for allowance except for the presence of claims 16-20 and 24-28, which are withdrawn, as well as claims 1, 4-9, 21, 29-33 and 36-42, which are currently under consideration but recite subject matter in the alternative with regards to SEQ ID NOS 1-9 and 17-159, which is subject matter drawn to non-elected inventions. Claims 1, 4-9, 21, 29-33 and 36-42 are therefore objected to as they contain subject matter drawn to inventions non-elected with traverse in the reply filed on 3/5/2002. Applicant is given ONE MONTH or THIRTY DAYS from this date of this letter, whichever is longer, to cancel the noted subject matter or take appropriate action (37 CFR 1.144). Failure to take action during this period will be treated as authorization to cancel the noted claims by Examiner's Amendment and pass the case to issue. **Extensions of time under 37 CFR 1.136(a) will not be permitted since this application will be passed to issue.***

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1 A portion of page 1 of the Decision on Petition dated February 27, 2006 reads: "*This letter is in response to the second renewed Petition under 37 C.F.R. § 1.144 and 1.181, filed 25 October 2005, requesting reconsideration of the Petition Decision of 30 August 2005, denying the first Petition requesting withdrawal of the restriction requirement to a single set of peptide nucleic acid (PNA) probes* (emphasis added). It is respectfully submitted that Applicant's Petition dated October 25, 2005 does not suggest that it seeks reconsideration under 37 C.F.R. § 1.144 but rather seeks review by the Deputy Commissioner for Patent Examination Policy under 37 C.F.R. §1.181 and M.P.E.P. § 1002.02(b) – i.e. a final agency decision.

(emphasis added; Office Action dated October 17, 2005 at page 2, paragraph 1)

Paragraph 2 of the action dated October 17, 2005 reads:

"2. *Claims 10, 13-15, 35 and 45 are allowed.*"[2]

(Id. at page 2, paragraph 2)

Accordingly, it is clear that allowable subject matter stands ready for issuance in the pending application but for the resolution of whether or not the restriction requirement was properly issued and applied thereby resulting in the present objection and related refusal to search or consider all of the presently pending claims on their merits.

The Decision on Petition dated February 27, 2006 further states:

> *"Applicants remain under obligation to reply to the Office action mailed 17 October 2005 within the time period set therein or as extended under 37 C.F.R. §1.136(a)."*
>
> (emphasis added; Decision dated February 27, 2005 at page 10)

It is respectfully submitted that the Office Action mailed on 17 October 2005 set a non-extendable period of 30 days for response. Applicant's options for response were limited. Either amend the claims as required by the Office else file a petition to challenge the validity of said restriction requirement which forms the basis for the "objection" and the related requirement to amend the claims. Since Applicant filed the Petition dated October 25, 2006, it is respectfully submitted that Applicant has answered the Office Action within the non-extendable time period. Accordingly, it is respectfully submitted that until the validity of the restriction requirement dated September 21, 2001

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2 At page 7, the Decision on Petition dated February 27, 2006 states: *"At this time, no claim is in condition for allowance in view of the outstanding objection that all claims encompass non-elected subject matter"*. Applicant respectfully submits that this statement conflicts with the record since apparently claims 10, 13-15, 35 and 45 are allowed. Clarification by the Office is requested.

is finally resolved, Applicant is under no obligation to take any further action in the subject application.

III. Petition History

In the above captioned application, the Examiner issued a restriction requirement in Office Action paper No. 9 (Office Action dated September 21, 2001). Applicant did enter traverse of the restriction requirement as well as make appropriate arguments and present a request for reconsideration, in reply to said Office Action, by submission dated January 18, 2002. Applicant did timely file a petition under 37 C.F.R. § 1.144 requesting review of the Examiner's decision by paper dated July 28, 2004 (the "First Petition"). Applicant received a Decision On Petition (the "First Decision") dated April 12, 2005 whereby Applicant's Petition was DENIED.

In response, Applicant filed a petition under 37 C.F.R. § 1.144 or 1.181 (the "Second Petition") on June 10, 2005 requesting reconsideration of the Examiner's decision with regard to a restriction requirement as set forth in Office Action paper No. 9 as well as for review of the First Decision. Responsive to said petition, Applicant received the Decision on Petition dated August 30, 2005 (the "Second Decision") wherein Applicant's petition was again DENIED.

In response, Applicant filed a petition under 1.181 (the "Third Petition") on October 25, 2005 seeking review of the Decision on Petition dated August 30, 2005 and a final agency decision. Responsive to said petition, Applicant received the Decision on Petition dated February 27, 2006 (the "Third Decision") wherein the Office failed to issue a final agency decision. This petition to the Deputy Commissioner for Patent Examination Policy is being timely filed within the two month period set for response to the Third Decision (See: the Third Decision at page 10). Accordingly, please consider this petition and issue a final agency decision.

For simplicity, this petition substantially reiterates the arguments set forth in the Third Petition. Any new arguments presented in the Third Decision (that were not presented in the First or Second Decision) will be addressed at the end of this paper in a separately entitled section.

IV. Preliminary Statement

The record reflects that claims 10, 13-15, 35 and 45 stand allowed. To avoid any confusion, Applicant reiterate that they have traversed the requirement that they: "*must amend his claims to include at least one claim limited to the elected invention in order for the claim objections to be withdrawn and for a claim* [presumably meaning the claims that are presently objected to] *to be placed in condition for allowance*" (Third Decision at page 6). The issue to be resolved is whether or not the remaining pending claims (i.e. claims 1, 4-9, 16-20, 21, 24-29, 29-33 and 36-42) must be amended to redact non-elected subject matter or whether the Office must consider the patentability of these remaining claims in their present format.

In support of the Office's action, the Office appears to take the position that each of the SEQ ID NOs 1-159 represents an independent and distinct invention and therefore, restriction under 35 U.S.C. § 121 is proper. However, Applicant maintains that the Office is confusing the concept of "misjoinder of invention" under 35 U.S.C. 121 and the related but distinct concept of "improper Markush grouping". Importantly, this erroneous analysis results in an absolute refusal by the Office to examine Applicant's claims on their merits and accordingly act as a *defacto* rejection of said claims.

V. Argument In Support Of Request To Withdraw The Restriction Requirement

a) The Subject Matter of the Present Controversy is Resolved by *In re Weber*

The Second Decision unequivocally states that the restriction requirement is based upon the Office's authority under 35 U.S.C. § 121. (Second Decision at page 1) *In re Weber* holds that:

> *"It is apparent that § 121 provides the Commissioner with the authority to promulgate rules designed to Restrict an Application to one of several claimed inventions when those inventions are found to be "independent and distinct". It does not, however, provide a basis to an examiner acting under the authority of the Commissioner to Reject a particular Claim on that same basis."*
>
> (*In re Weber*, 580 F.2d 455, 458, 198 U.S.P.Q. 328, 331-332 (CCPA, 1978))

"We hold that a rejection under § 121 violates the basic right of the applicant to claim his invention as he chooses." (emphasis added).

(*Weber at* 459, 198 U.S.P.Q. at 332 (CCPA, 1978))

Accordingly, it is clear from *In re Weber* that the legal issue of whether or not the Office may impose a restriction requirement and refuse to examine subject matter within the scope of the claim so restricted has been decided against the Office. It is well settled that such requirements violate 35 U.S.C. § 112, where the applicant is statutorily entitled to claim his invention as he deems proper, notwithstanding 35 U.S.C. § 121. **This is true whether or not the single claim comprises two or more independent and distinct inventions.**

The restriction requirement dated September 21, 2001 states: *"Restriction to one of the following inventions is required under 35 U.S.C. § 121"*, and then proceeds to list 13 Groups defined by the same claims (i.e. Claims 1-15, 21-23 and 29-45 – Groups I-V, VII, IX and XI-XVI) and 5 Groups having substantial claim overlap with the previously described 13 Groups (i.e. Groups VI, VIII, X, XVII and XVIII; Office Action dated September 21, 2001 at pages 2-5). Thus, it is clear that the restriction requirement attempts to restrict subject matter within a single claim.

The restriction requirement asserts that these Groups define distinct inventions (paragraph 2 of the September 21, 2001 Office Action at pages 5-6). Consequently, the Examiner concludes that the restriction requirement is proper under 35 U.S.C. § 121, 37 C.F.R. § 1.142 (a) and 37 C.F.R. § 1.141(a). *Id.* at page 6. Importantly, the rules of practice require Applicant to make an election when responding to the restriction requirement whether or not the restriction requirement is traversed (37 C.F.R. §1.142, 1.143, 1.145 & 1.146), which it was (See: Applicant's response dated January 18, 2002). Consistent with its present actions and its interpretation of the rules of practice, the Office has never considered the patentability of Applicant's claims as filed but has repeatedly imposed an absolute requirement that Applicant redact non-elected subject matter from the claims as originally filed (e.g. See: "Office Action dated April 23, 2003 at paragraphs 1-2, pages 2-3; the Office Action dated January 29, 2004 at paragraph 1, page 2; the Office Action dated April 27, 2005 at paragraph 8, page 5; the concluding remark of the Second Decision (page 6) and the Office Action dated October 17, 2005 at page 2, paragraph 1).

This is precisely the situation that *In re Weber* and *In re Haas* prohibit. That is, even if the Office determines that a single claim defines two or more distinct inventions, it may not issue a restriction requirement as to subject matter within a single claim coupled with an objection that denies consideration of the subject matter of that claim on this basis and thereby *de facto* reject the claim. In the present case the Office characterizes its actions as an "objection", not a "rejection", but its action has the same effect. In essence, whether characterized as an "objection" or a "rejection", both cases ***"violate[s] the basic right of the applicant to claim his invention as he chooses"*** *(In re Weber, 580 F.2d 455, 459, 198 U.S.P.Q. 328, 332, (CCPA, 1978)).* Specifically, the Office refuses to consider the patentability of Applicant's claims as filed and absolutely requires that they be amended to redact non-elected subject matter. Since the actions of the Office are clearly contrary to established precedent, it is respectfully requested that issuance of the restriction requirement and related objection is based upon clear error and accordingly they should be withdrawn.

b) Reliance Upon The M.P.E.P. Cannot Cure Faulty Analysis

The Second Decision acknowledges that: *"Applicants* [sic] *are correct that the Office must follow statute and judicial precedent"*. (Second Decision at page 1) Consequently, nothing in the M.P.E.P. will cure the Office's failure to follow *In re Weber* or *In re Haas*. In particular, references to the M.P.E.P. dealing with cases involving "improper Markush grouping" are irrelevant and off point. Applicant comments on the arguments in the First Decision and Second Decision as follows:

(i) M.P.E.P. §803.02

In the First Decision it was argued, after quoting from M.P.E.P. § 803.02, that: *"The 159 sequences recited in claim 1 of the instant application do not share a common utility nor do they share any substantial structural feature, let alone any substantial feature disclosed as being essential to that utility"*. (emphasis added; First Decision at page 3) Later in the First Decision the Office argues:

> *"Applicants'*[sic] *attention is again directed to the MPEP section 803.02 which deals with the* ***treatment of Markush-Type claims*** *which list alternatives having a common core structure and function:*
>
> *If the members of a* ***Markush group*** *are sufficiently few in number or so closely related that a search and examination of the entire claim can be made without serious burden, the examiner must examine all the members of the Markush group in the claim on the merits, even though they are directed to independent and distinct inventions."*

(emphasis added: First Decision at page 4)

The point the Office appears to be making here is that its actions were appropriate based upon a finding that the claims lack "unity of invention" and therefore the claims contain an "improper Markush grouping". However, the Second Decision specifically states that: *"Because no claim in this application has been rejected for containing an improper Markush group, the argument the Office's actions are inconsistent with the holding of In re Harnisch is not persuasive."* (Second Decision at page 2) Consequently, the Second Decision appears to confirm Applicant's belief that Office's reliance on M.P.E.P. § 803.02 in the First Decision is "off point and irrelevant". Importantly, the decision *In re Harnisch* specifically states that:

> *"It should also be clear from what we have said that we adhere to our holdings in In re Weber, supra, and In re Haas (Hass II), supra. Nothing we have said herein is intended to change or modify them in any way; nor do we think anything said could be reasonably construed to have such an effect."*

(*In re Harnisch*, 631 F.2d 716, 722, 206 U.S.P.Q. 300, 305 (CCPA, 1980)

Consequently, it is clear that the Office's reliance on M.P.E.P. § 803.02 and the decision in *In re Harnisch* will not cure its failure to comply with *In re Weber* and *In re Haas* which decisions specifically prohibit the Office from acting under 35 U.S.C. § 121 to thereby impose a restriction requirement within a single claim coupled with an objection that constitutes a refusal to consider subject matter within that claim. Even under the practice sanctioned in MPEP 803.02, which addresses the possibility of multiple inventions within a single claim, examiners are instructed to first examine the elected invention and if it is determined to be patentable, to continue the search and

examination of other subject matter within that claim to determine whether the subject matter in the claim as a whole is patentable. M.P.E.P § 803.02. Thus, apparently the Office has not followed its own protocols in this case.

(ii) M.P.E.P. § 2175.03(h)

In the Second Decision the Office argues: "*Applicant also point to In re Harnisch and MPEP 2173.05(h) to argue that the proper test for whether SEQ ID NOs 1-159 are **distinct** is whether they share a common utility.*" (emphasis added in **bold**, underlined text was originally in *italic*, Second Decision at page 3) It is respectfully submitted that this mischaracterization of Applicant's arguments illustrates another fault with the Office's position. Applicant has argued that it is irrelevant whether or not SEQ ID NOs 1-159 are distinct because *In re Weber* holds that it is improper for the Office to restrict one or more inventions within a single claim and refuse consideration of subject matter within the scope of that claim to determine whether it is patentable regardless of whether or not they are "**independent and distinct**". The argument set forth in the Second Decision clearly being based upon an incorrect premise renders its related arguments and conclusion to be "irrelevant".

(iii) M.P.E.P. § 808

At page 5 of the Second Decision it has been argued:

"MPEP 808 sets forth further guidance for insisting upon restriction:

> *Every requirement to restrict has two aspects: (A) the reasons (as distinguished from the mere statement of conclusion) why the inventions as claimed are either **independent or distinct**; and (B) the reasons for insisting upon restriction there between as set forth in the following sections.*

*The arguments appear to be directed to the elected invention, probes comprising SEQ ID NOs 10-16. These arguments are not commensurate with the invention as claimed. MPEP 808 explains that it is the invention, as claimed, which is considered for **distinctness and independence**."*

(Second Decision at page 5)

It is clear from the forgoing that again the Office's arguments pertaining to M.P.E.P. § 808 focus on determining when an invention is independent or distinct. However, since the present restriction requirement attempts to apply a restriction within a single claim under 35 U.S.C. § 121 and deny consideration of subject matter within the scope of that claim, this analysis is again "off point and irrelevant". This is clear since *In re Weber* and *In re Haas* specifically hold that the Office may not restrict within a single claim under 35 U.S.C. 121 and deny consideration of subject matter within the scope of that claim regardless of whether or not it claims two or more inventions that are "independent and distinct". *(In re Weber, 580 F.2d 455, 458, 198 U.S.P.Q. 328, 332, (CCPA, 1978))*

(iv) M.P.E.P. § 803.04

At page 6 of the Second Decision, it is stated that:

> *"MPEP 803.04 explains how claims directed to polynucleotide sequences claimed both individually and in sets will be restricted and examined."*
>
> (Second Decision at page 6)

The Second Decision then goes on to explain how the Office supposedly applied M.P.E.P. § 803.04 to the present claims based upon the presumption that restriction is proper.[3] However, it is self-evident that *In re Weber* and *In re Haas* prohibit the Office from restricting, for example, SEQ ID NOs. 1-159 within claim 1 under 35 U.S.C. § 121 coupled with an objection that denies consideration of subject matter within the scope of claim 1. Consequently, this argument is again "irrelevant and off point" since no such presumption is valid.

(v) Summary

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3 Since M.P.E.P. § 803.04 appears to absolutely require that single claims comprising 10 or more different nucleotide sequences be restricted, the section is clearly in conflict with *In re Weber* and *In re Haas*. Since the Office has acknowledged that they must comply with binding precedent (Second Decision at page 1), reliance on this section of the M.P.E.P. is clearly improper.

In summary, it is clear that like the First Decision before it, the Second Decision confuses the concepts of misjoinder of invention under 35 U.S.C. § 121 with the related but distinct concept of "improper Markush grouping". Moreover, both the First Decision and the Second Decision are based upon an incorrect presumption that it is permissible for the Office to restrict within a single claim under 35 U.S.C. § 121 and thereby deny consideration of subject matter within the scope of that claim. Because conclusions that are based upon incorrect premises are inherently flawed, it is clear that the Office's improper reliance on this incorrect premise and the M.P.E.P. cannot cure the faulty analysis of the First Decision and the Second Decision. Accordingly, reconsideration and withdrawal of the Restriction Requirement and related objection are respectfully requested.

c) Applicant Reiterates That "Unity of Invention" Exists

In support of the First Decision and the Second Decision, the Office repeatedly has quoted from the M.P.E.P., which itself often makes reference to *In re Harnisch*, 631 F.2d 716, 206 U.S.P.Q. 300 (CCPA, 1980) and *Ex Parte Hosumi*, 3 U.S.P.Q.2d 1059 (Bd. Pat. App. & Int. 1984), with an emphasis on attempting to show that there is no common utility[4] to the 159 different PNA probes claimed in, for example, claim 1. For example, the Second Decision argued:

> *"As explained in the previous decision, the 159 peptide nucleic acid sequences recited in claim 1 of the instant application do not appear to share a* ***common utility*** *nor do they share any* ***substantial structural feature****, let alone any* ***substantial feature disclosed as being essential to that utility****. Probes which bind to common structure, such as a selected chromosome, are not required to share a common structure. This is supported by the comparisons of SEQ ID Nos 10-16, which appear to share no significant structure in common and yet hybridize to Chromosome Y. This is because the probes hybridize to different regions of Chromosome Y. This is the case in the instant application and the Office has grouped the probes based on their specific chromosome binding affinity (i.e. binding to chromosomes X, Y, 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 16, 17, 18 or*

4 In *Harnisch* the court discussed the use of "a single structural similarity" as being the appropriate test since *"in any Markush group the compounds "will differ from each other is certain respects"* and the court used the *"unity of invention""* criteria to establish whether or not *"unrelated inventions are involved"*. *In re Harnisch*, 631 F.2d 716, 722, 206 U.S.P.Q.2d 300, 305-306 (CCPA, 1980)

20, etc...) and thus every invention has been placed within a group which has a common utility.

MPEP 2175.03(h) states in part that:..."

(Second Decision at page 4, emphasis added)

As previously discussed, that line of reasoning is irrelevant and off point since the restriction requirement is based upon misjoinder of invention under 35 U.S.C. § 121 and not based "improper Markush grouping"[5]. Regardless, Applicant disagrees with the analysis and conclusion. Specifically that there is no common function (utility) to the 159 peptide nucleic acid probes claimed in, for example, claim 1 or that, by implication, there is no "unity of invention" thereby leading to the conclusion that an improper Markush group has been claimed by Applicant.

In re Harnisch dealt with a Markush claim (and related dependent claims) to "coumarin compounds" that were admittedly all "dye stuffs"[6]. Thus, all the "coumarin compounds" had an asserted common function (utility) that was identified in the specification. In *Harnisch*, the examiner had rejected the claims under 35 U.S.C. § 121 but the Board of Patent Appeals and Interferences (BPAI) summarily reversed that rejection citing to *In re Weber* and *In re Haas*. *In re Harnisch* at 717, 206 U.S.P.Q at 301. However, the BPAI rejected the claims as being "drawn to improper Markush groups" (i.e. the inventions were unrelated). *Id.* at 717-718, 206 U.S.P.Q. at 301. The BPAI reasoned:

"Applying the facts of this case to the principles enumerated, we find that the members of the Markush groups of the claims do not belong to a known or recognized genus and possess widely different physical or chemical properties. Aside from the obvious fact that the compounds encompassed by the claims are not functionally equivalent, said compounds, considered as a whole, are so dissimilar and unrelated chemically or physically that it would be repugnant to accepted principles of scientific classification to associate them together as a generic group. For example, the types of derivatives encompassed by the

5 The Office had previously stated that Applicant's arguments are not persuasive because the claims are not rejected for improper Markush grouping (See The Second Decision at page 2) but now seems to imply that the claims use "improper Markush grouping". Thus, it appears that the Office takes both sides of this issue.

6 The *Harnisch* court seemed to rely heavily on the admission of the solicitor in its opinion. See: *In re Harnisch*, 631 F.2d 716, 719, 206 U.S.P.Q. 300, 303 (CCPA, 1980)

> *Markush claim may include polyfused N-heterocyclics, cyclic, acyclic and aromatic amines, sulfonamides, phthalimides, quarternary ammonium salts, phosphorus heterocyclics, phosphates, aldehydes, azomethines, hydrazones, ethers, esters, halogens, alcohols, nitriles, piperidines, furanes, pyrroles, indoles, amongst others. It is clear on the record the involved compounds cannot be considered functionally equivalent, in fact some being no more than intermediates for the others. ... The mere fact that there is* ***a single structural similarity (i.e., the coumarin group) in not in itself sufficient reason to render all the embodiments functionally equivalent, particularly when the ultimate properties of the final products would not be expected to possess any recognized functional relationship.***"

(*Id.* at 718, 206 U.S.P.Q. at 302, emphasis added in bold, underlined text in *italic* in the original)

Notwithstanding this characterization of the claims, the court in *Harnisch* found that the BPAI had erred for not recognizing that all of appellant's claimed compounds were dye stuffs (i.e. the common function) having a "single structural similarity" (i.e. the coumarin group), therefore possessed "unity of invention" (i.e. the inventions were related). *Id.* at 722, 206 U.S.P.Q. at 305.

As argued by Applicant in the Second Petition, in determining what constitutes "common function", *In re Harnisch* expressly states:

> *"Over thirty years ago this court decided In re Jones, 34 CCPA 1150, 162 F.2d 479, 74 USPQ 149 (1947), reversing an "improper Markush group" rejection of claims to chemical compounds which were [*722] growth-regulating compositions for plants, fungicides, and insecticides.* ***Notwithstanding their various properties, the court found all of the compounds included in the claims were plant growth stimulants, thus having a common function.*** *The court noted that in any Markush group the compounds "will differ from each other in certain respects." It laid down the proposition, with which the PTO agrees in its M.P.E.P., that* ***in determining the propriety of a Markush grouping the compounds must be considered as wholes and not broken down into elements or other components."*** *(emphasis added)*

(*In re Harnisch*, 631 F.2d 716, 722, 206 U.S.P.Q. 300, 305 (CCPA, 1980)

From the foregoing it is clear that The Office should not, when considering the appropriateness of Markush groupings, **focus on trivial distinctions of elements, or other components, of the claimed compounds but must consider the compounds, and**

their associated functions, as a whole with an emphasis on determining whether or not the members of the Markush group all possess a single structural similarity and common function (i.e. they are related inventions).

This position seems to also be supported by M.P.E.P. § 2173.05(h), which reads:

> *"The materials set forth in the Markush group ordinarily must belong to a recognized physical or chemical class or to an art-recognized class. However, when the Markush group occurs in a claim reciting a process or a combination (not a single compound), it is sufficient if the members of the group are disclosed in the specification to possess at least one property in common which is mainly reasonable for their function in the claimed relationship, and it is clear from their very nature or from the prior art that all of them possess this property." (emphasis added)*
>
> (M.P.E.P. § 2173.05(h)

In the present case, the Examiner, as well as the First Decision and the Second Decision, focuses on trivial distinctions associated with elements, or other components, of PNA (i.e. the nucleobases) and not on the compounds and their associated structural similarity and common function as described in the specification, when considered as a whole. That is the nature of the asserted error.

For example, the Second Decision reasons:

> *"As set forth in the previous decision, with respect to the nature of the invention, the claimed probes are not traditional nucleic acids, they are PNA or Peptide-Nucleic acids and have been claimed as "Non nucleic acid probes."[7] The difference with a PNA is that the backbone is not a traditional sugar-phosphate nucleic acid backbone, but one that has peptide structures. PNA's function like nucleic acids in that they contain a sequence of bases (usually traditional nucleotide bases) (what is termed in the claims as a probing nucleobase sequence) which is responsible for the hybridization of a PNA to DNA.*[8]
>
> (Footnotes added; Second Decision at page 3)

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7 All claims have now been limited to peptide nucleic acid probes so this distinction is no longer relevant.

8 This statement alone is recognition by the Office that peptide nucleic acids represent an art recognized class of molecules (i.e. have a single structural similarity recognized in the art).

Thus, the Second Decision continues, like the First Decision, to focus on nature of the nucleobases linked to the peptide nucleic acid backbone and therefore fails to consider how these various claimed PNA probes are related with respect to their structural similarity and common function in the context of the specification when viewed as a whole. In particular, the restriction requirement, the First Decision, the Second Decision all fail to recognize that the 159 different PNA probes are all PNA probes (i.e. single structural similarity) and all hybridize to human chromosomes (i.e. the common function) and thereby can be used, alone or in combination, to determine human chromosomes and/or distinguish between different human chromosomes (i.e. evidence of related inventions). For example the specification reads:

> *"The non-nucleic acid probes of this invention are suitable for detecting, identifying or quantitating human chromosomes X, Y, 1, 2, 3, 4, 6, 7.8, 9, 10, 11, 12, 16, 17, 18 or 20, as well as 13/21 as a pair, in a sample or in the individual cells of the sample."*
>
> (Specification at page 21, lines 2-5)

That common function was also demonstrated in specification by the Examples and illustrated in the Figures. For Example, Figures 12A and 12B illustrate the simultaneous determination of chromosomes X, Y and 1 using a mixture of the claimed PNA probes. All other of the 159 PNA sequences were likewise shown to be suitable for the analysis of human chromosomes (c.f. Example 9 of the specification and the related Figures). It is self-evident that the 159 PNA probes can be mixed in other ways to perform similar assays for the determination of the same and/or other chromosomes[9],

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9 The specification expressly states: *"In preferred embodiments, non-nucleic acid probes are organized into a set that is designed to detect, identify or quantitate, individually or together with other chromosomes, each of chromosomes X, Y, 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 16, 17, 18 and/or 20, as well as 13/21 as a pair, that may be present in the sample. In a most preferred embodiment, a probe set is suitable for the specific detection, identification and/or quantitation of the total number of each of human chromosomes X, Y, 1, 2, 3, 4,6, 7, 8, 9, 10, 11, 12, 16, 17, 18 and/or 20, as well as 13/21 as a pair, in a sample of interest. Preferably, the probes or probe sets are integrated into an assay used for the simultaneous detection, identification and/or quantitation of some or all human chromosomes."* (emphasis added)

such as for example their use in a prenatal assay (Specification at page 11, lines 9-17). Thus, in the present application, it is clear that all of the PNA probes are PNA probes (i.e. single structural similarity) and all of SEQ ID Nos. 1-159 can be used to determine human chromosomes and/or distinguish between different human chromosomes (i.e. they all possess a common function that is expressly described in the patent specification).

Interestingly, the Second Decision asserts that a PNA probe of SEQ ID No: 10 could not be substituted for a PNA probe of SEQ ID No. 9 because the PNA probe of SEQ ID No: 10 will hybridize to human chromosome Y while SEQ ID No. 9 will hybridize to human chromosome X. (Second Decision at page 4) While true, this distinction is off-point.

In *Harnisch* the BPAI attempted to distinguish between compounds that were "dye stuffs" (a common function) and compounds that were intermediates to "dye stuffs" thereby suggesting that this distinction was a critical difference. *In re Harnisch* at 717-718, 206 U.S.P.Q. 300 at 301-302. In *Harnisch*, it was determined that all of the "coumarin compounds" (i.e. single structural similarity) were dye stuffs (i.e. common function); albeit true that some were intermediates (a subgenus) to other coumarin compounds. This was true regardless of the fact that they "*may include* [diverse appended groups such as] *polyfused N-heterocyclics, cyclic, acyclic and aromatic amines, sulfonamides, phthalimides, quarternary ammonium salts, phosphorus heterocyclics, phosphates, aldehydes, azomethines, hydrazones, ethers, esters, halogens, alcohols, nitriles, piperidines, furanes, pyrroles, indoles, amongst others*" *Id.* at 718, 206 U.S.P.Q. at 302. Thus, the BPAI's focus on an asserted subgenus (not the genus) and the appended groups (as compared with analysis of the compounds as a whole) was its error.

In the present case, by focusing on various disclosed subgenera (i.e. groups of PNA probes for various individual chromosomes) and the appended nucleobases but not on the genus disclosed in the specification (i.e. PNA probes for the analysis of "**some or all human chromosomes**") and the PNA probes as a whole, the Office makes an error that is remarkably similar to that found in *Harnisch*. Thus, Applicant submits he has claimed many related PNA probe compounds that make up a proper Markush group as did the many "coumarin compounds" of *Harnisch*. *In re Harnisch* at 722, 206 U.S.P.Q. 300 at 305-306. Consequently, Applicant reiterates that "unity of invention" exists within the

159 PNA probes claimed, for example, in claim 1 because all the probes can be used alone or in combination to determine human chromosomes and/or distinguish between different human chromosomes as more fully described in the specification, examples and figures.

VI. Rebuttal To New Arguments Presented in the Third Decision

(i) Preface

Applicants submit that arguments made in the Third Decision (including the First Decision & Second Decision) infer that there is no legal distinction between misjoinder of invention under 35 U.S.C. § 121 and "improper Markush grouping". Applicants disagree and point to the distinctions made between *Weber* and *Haas* as compared with the decision in *Harnisch*. Applicants submit that the present restriction requirement, which has resulted in the present "objection" and the related *defacto* rejection of Applicants claims, is clearly based upon misjoinder of invention under 35 U.S.C. § 121 and not on "improper Markush grouping". Accordingly, all discussion of "unity of invention" and related topics demonstrate that the restriction requirement dated September 21, 2001 is *prima facia* defective for its failure to specifically state that the restriction requirement was made for "improper Markush grouping" rather than for misjoinder of invention under 35 U.S.C. § 121. For this reason alone the restriction requirement should be withdrawn. Applicant further reiterates that since the Office contends that the present restriction requirement was made under 35 U.S.C. § 121 and required restriction within a single claim, *Weber* and *Haas* are the controlling precedent and necessitate withdrawal of the restriction requirement dated September 21, 2001.

(ii) Rebuttal to Section (1) of the Third Decision

In the Third Decision, the Office finally acknowledges that for a Markush-type claim, more is required of the Office than a mere finding of independent and distinct inventions in order for it to require restriction within a single claim (Third Decision at page 5). However, for reasons that are not self-evident, the Office has misstated the test for determining whether a Markush group is proper or not. Specifically, the Third Decision states:

> *"Further, as indicated above in MPEP 803.02, the Office must additionally show that the inventions lack unity of invention, which requires the Office to establish that either there is a lack of common utility or a lack of substantial structural feature essential to that common utility."*

(bold text original, emphasis added in underline; Third Decision at page 5)

However, *Harnisch* clearly articulates that the elements of "unity of invention" require "single structural similarity" and "common function" not "substantial structural feature" and "common utility"[10]. *In re Harnisch* at 722, 206 U.S.P.Q. 300 at 305-306. Accordingly, it is clear that the Third Decision is applying an improper test to determine whether or not improper Markush grouping is present. Since they rely upon the application of an improper test, the conclusions of the Third Decision are clearly flawed.

That unity of invention exists, is evident from the argument set forth in section V(c) above. Moreover, Applicant will address the unity of invention issue again with respect to Section 3 of the Third Decision, discussed below.

(iii) Rebuttal to Section (2) of the Third Decision

To the assertion that the Examiners are required to follow the M.P.E.P., it suffices to say that the Office has acknowledged that the M.P.E.P. must follow binding precedent (First Decision at page 3). Accordingly, Applicants reiterate that, for reasons argued, it is believed that the Office has failed to follow the decisions set forth in *Weber*, *Haas* and/or *Harnisch* and that the Office's asserted reliance on the M.P.E.P. cannot cure this fault.

Moreover, MPEP § 803.04 assumes that unity of invention does not exist – a point not conceded by Applicant. Because unity of invention exists for the pending claims, the Office's reliance on MPEP§ 803.04 if fatally flawed. It should also be noted that the Office's application of MPEP§ 803.04 conflicts with the more appropriate application of MPEP § 803.02 which (as noted above), instructs examiners to first examine the elected invention and if it is determined to be patentable, to continue the search and examination of other subject matter within that claim to determine whether the subject

10 It is presumed that the Office intends "common utility" to mean "common function" as described in *Harnisch*. If not, it is requested that the Office clarify its intent.

matter in the claim as a whole is patentable. M.P.E.P § 803.02. Thus, it seems that the Office has failed to follow its own procedures.

(iv) Rebuttal to Section (3a) of the Third Decision

In this section of the Third Decision, the Office reproduces information from the Sequence List (See 37 C.F.R. § 1.821-1.825). Applicants reiterate that the Office's reliance on M.P.E.P. § 2175.03 (b) and reference to only the one letter codes of the nucleobases (i.e. a, c, g or t) listed in the sequence list is obvious error. This approach defies the decision in *Harnisch* by failing to consider whether or not there is a "single structural similarity" between members of the Markush group based upon an analysis of the compounds considered as a whole and not piecemeal (i.e. "*not broken down into [nucleobase] elements or other components*"). *Id.* Since the conclusions of the Third Decision rest upon an improper analysis, they are flawed.

Additionally, Applicant wishes to comment on the statement:

> *"The fact that the backbone of a nucleic acid or nucleic acid analog may vary so widely, e.g. from a polyanionic ribose phosphate backbone to a neutral polypeptide backbone emphasizes the lack of specificity of the backbone."*
>
> (Third Decision at page 8)

The problem with the Office's reliance upon this statement is threefold. First, the claims at issue relate only to PNA probes so the discussion of other backbone choices is irrelevant and off-point. Second, as previously stated, this analysis emphasizes the nucleobase element and fails to recognize the well-established importance of the PNA backbone on specificity and hybridization properties of PNA oligomers and thus fails to consider the compositions as a whole (See: Egholm et al., *Nature*, 365: 566-568 (1993) & Nielsen et al., *Chemical Society Reviews*, 73-78 (1997) both attached). Third, this analysis fails to consider that these probes possess a "single structural similarity" since they all comprise purine and pyrimidine nuclebases that are linked to the "PNA" backbone. Thus, Office's analysis is flawed at various levels.

(v) Rebuttal to Section (3b) of the Third Decision

At page 9, the Third Decision reads: *"It is clear that the critical issue in this restriction controversy is whether or not the inventions embraced by claim 1 in particular possess Unity of Invention."* Applicant disagrees since the original restriction requirement fails to assert that restriction is based upon "improper Markush grouping". Regardless, Applicant concedes that if "improper Markush grouping" were present, the Office could require restriction within a single claim. However, for the reasons described above, it is believed that claim 1 (and the other claims to which an objection applies) passes the test for Unity of Invention as articulated in *Harnisch*. Moreover, Applicant reiterates that the Office has failed to apply the proper test and that may be, in part, why there is a disagreement about, for example, whether or not claim 1 uses proper Markush format.

At pages 8-9 of the Third Decision, the Office attempts to discredit the argument that the specification and figures demonstrate the "common function" of the PNA probes. With reference to only the brief description of Figure 12, and not the entire specification and examples (i.e. the Office takes a snippet of text out of context), the Office asserts that:

> *"It is not clear whether applicant is arguing that an individual probe for chromosome X would also specifically bind chromosome Y and 11."*
>
> (Third Decision at page 9)

The Office then states:

> *"Table 1 does not disclose any individual probe which binds chromosomes X, Y and 11".*
>
> (Third Decision at page 9)

With reference to Example 10 on page 60-61 of the specification, there is a more complete description of the experiment with reference to color Figures 12A and 12B. As described, the sample used in Figure 12A is a female having two X chromosomes that are visible by the two orange dots. In Figure 12B the sample used belongs to a male since there is one X chromosome (orange dot) and one Y chromosome (green dot). The two red dots in each of Figures 12A and 12B illustrate two chromosomes 1[11] (not

11 As is discussed in the specification (and well known in the art) in the "normal" state, humans have pairs of chromosomes.

chromosome 11 as asserted by the Office in the Third Decision[12]). Further analysis of the discussion in Example 10, clarifies the issue that the Office has asserted is unclear from its cursory reference to the brief description of the drawings. Specifically, it is clear from Example 10 and from Table 1, that each of the different PNA probes used hybridized to a particular chromosome but that when used to together in a single assay, it is possible to independently identify and quantify different human chromosomes. This fact has not been disputed (or even referred to) by the arguments set forth in the Third Decision. Because all of the PNA probes can be used in this manner, they represent related inventions according to the criteria set forth in *Harnisch*.

Finally, at the end of the Third Decision, the Office suggests that in the 27 years since the CCPA decided *In re Weber* there has been an unforeseeable search burden thrust upon the Office (Third Decision at page 10). In brief, such an argument is neither relevant, nor is it believed to be accurate. In addition to not supplying any evidence of such increased burden, Applicant submits that the search burden is exactly as it stood after *Weber*, *Haas* and *Harnisch* and that the advent of computer searching has made the job of searching for relevant art much easier. That of course is why 37 C.F.R. § 1.821-1.825 requires applicants to submit electronic sequence listings.

In summary, it is further disputed that the present claimed invention lacks "unity of invention" for the reasons argued. Although not relevant since the Office is obligated to follow precedent, it is disputed that searching, for example claim 1, imposes an undue burden on the Office, whether or not unity of invention exists.

VII. Summary

It is respectfully submitted that based upon the foregoing arguments, it is self-evident that the restriction requirement and related objection are flawed. Specifically, the restriction requirement and related objection conflict with the express holding of *In re Weber* and *In re Haas*. Although it is acknowledged that the Office classifies its present action as an "objection" and not a "rejection" of the claims, it is equally clear that this is a distinction without a difference. Specifically, in both cases Applicant is required to

12 This mistake in the Third Decision is further evidence that the Office has failed to consider the teachings of the specification as a whole. This is an example of how piecemeal analysis leads to confusion of significant facts and evidence.

redact non-elected subject matter within a single claim so that all claims have never been, and will never be, examined on their merits. Thus, the Office's actions are clearly inconsistent with *In re Weber* and *In re Haas* and thereby constitute a *de facto* "rejection". Applicant further submits that the presently pending claims use proper Markush grouping but that, in any event, this is immaterial given the basis for the restriction requirement and related objection. Since the Office must follow statute and judicial precedent, the present "objection" is clear error. Withdrawal of the restriction requirement and related "objection" to claims 1, 4-9, 16-20, 21, 24-28, 29-33 and 36-42 of the present application is therefore respectfully requested or in the alternative, issuance of an adverse final agency decision that can be appealed to the District Court is requested.

Respectfully submitted
On behalf of Applicant,

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PNA hybridizes to complementary oligonucleotides obeying the Watson–Crick hydrogen-bonding rules

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DNA ANALOGUES are currently being intensely investigated owing to their potential as gene-targeted drugs[1–3]. Furthermore, their properties and interaction with DNA and RNA could provide a better understanding of the structural features of natural DNA that determine its unique chemical, biological and genetic properties[3,4]. We recently designed a DNA analogue, PNA, in which the backbone is structurally homomorphous with the deoxyribose backbone and consists of *N*-(2-aminoethyl)glycine units to which the nucleobases are attached[5–9]. We showed that PNA oligomers containing solely thymine and cytosine can hybridize to complementary oligonucleotides, presumably by forming Watson–Crick–Hoogsteen $(PNA)_2$–DNA triplexes, which are much more stable than the corresponding DNA–DNA duplexes[5–7], and bind to double-stranded DNA by strand displacement[5,8]. We report here that PNA containing all four natural nucleobases hybridizes to complementary oligonucleotides obeying the Watson–Crick base-pairing rules, and thus is a true DNA mimic in terms of base-pair recognition.

A non-self-complementary pentadecamer, PNA, was designed with a GTAC sequence in the middle and containing an almost equal number of pyrimidines and purines, but having no more than two purines or pyrimidines juxtaposed. The thermal stability, measured as the melting temperature, T_m, of complexes between the pentadecamer H-TGTACGTCACAACTA-NH_2, and the complementary deoxyoligonucleotide 3′-d(ACATGCAGTGTTGAT) (termed antiparallel orientation: amino terminal of the PNA facing the 3′-end of the oligonucleotide), was 69.5°C (Table 1), whereas the T_m of the corresponding PNA-DNA complex in the parallel orientation was 56.1 °C. The T_m for the PNA-RNA complexes was 72.3 and 51.2 °C, respectively (Table 1). The orientation preference was further settled by using two decamer PNAs, and hybridizing these to complementary oligonucleotides in both orientations. The antiparallel orientation was preferred in all cases (Table 1). Furthermore, note that virtually identical T_ms were obtained regardless of which strand is the PNA and which is the DNA (Table 1, and columns 2 and 3). The results also show that the presence of the terminal lysine amide, which was contained in our original PNA to reduce aggregation of oligothymine PNA[5,6], does not influence the preferred orientation of the PNA relative to the DNA.

When a Watson–Crick base-pair mismatch was introduced in the oligonucleotide at any position facing the four middle PNA nucleobases (GTCA) in the pentadecamer, a large decrease in T_m (8–20 °C; Fig. 1) was observed, thereby providing compelling evidence that PNA–DNA recognition takes place by Watson–Crick base pairing, that is, A·T and G·C base pairing. For comparison we also measured the thermal stabilities of the corresponding DNA–DNA duplexes (Fig. 1). Note that for virtually all base-pair mismatches, the decrease in thermal stability is greater for the PNA–DNA complex than for the DNA–DNA complex, suggesting (provided that the differences in transition enthalpies are identical) that the sequence discrimination is, if anything, more efficient for PNA recognizing DNA than for DNA recognizing DNA.

The unambiguous evidence for Watson–Crick base pairing suggests that these PNA–DNA complexes are duplexes rather than the $(PNA)_2$–DNA triplexes previously observed with homopyrimidine PNA[6,7]. This conclusion was confirmed by titration experiments using ^{32}P-labelled oligonucleotides in a gel retardation assay (Fig. 2*a*) or using circular dichroism (CD) for the detection of complex formation (data not shown).

FIG. 1 Thermal stabilities of PNA·DNA complexes. *a*, Chemical structure of PNA and DNA. B is the nucleobase, and R^1 = H, or lysinyl amide for the PNAs discussed here. The PNAs are written from the N to the C terminal using normal peptide conventions: H, a free amino group; $-NH_2$, a terminal carboxamide. The PNAs were synthesized as previously described[6–8]. They were purified by HPLC and their composition was verified by mass spectrometry. *b*, Effect of base-pair mismatches on the thermal stability of PNA–DNA complexes. The thermal stability of complexes between PNA, H-TGTACGTCACAACTA-NH_2 and the 13 oligonucleotides 3′-d(ACATGXYZWGTTGAT), in which X, Y, Z, W = C, A, G, T for the case in which the PNA and DNA sequences are complementary. In each of the 12 other oligonucleotides, three of the bases (X, Y, Z, W) were complementary and the fourth was one of the three non-complementary nucleobases. For example, when X = T, Y = G, Z = A, then W = A or C or G and so on. Thus each of the 12 oligonucleotides contains one of the 12 possible base-pair mismatches relative to the PNA pentadecamer. The T_ms of these complexes are displayed as solid bars. For comparison, the results of similar experiments with DNA–DNA duplexes are shown (hatched bars). *c*, Effect of ionic strength on the thermal stability of PNA·DNA (○) or DNA·DNA (●) complexes.
METHODS. Hybridizations were done in 10 mM sodium phosphate, pH 7.0, 150 mM NaCl, 1 mM $MgCl_2$. Qualitatively similar results were obtained using oligonucleotides in the parallel orientation.

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Information about secondary structure may be obtained from CD measurements because these are sensitive to the base-pair geometry in the helix. Figure 2*b* shows the CD spectra of the PNA-DNA, PNA-RNA, DNA-DNA and DNA-RNA duplexes. The CD spectra of DNA-DNA and antiparallel PNA-DNA and PNA-RNA duplexes are largely similar, suggesting that these PNA-DNA and PNA-RNA duplexes are right-handed helices with a base-pair geometry not much different from that found in a B- or an A-form DNA helix. But it is interesting that the CD spectra, and thus the structure, of the parallel versus antiparallel PNA-DNA (or PNA-RNA) duplexes are distinctly different.

Thermodynamic parameters for hybridization were extracted from thermal stability measurements[10,11] to determine ΔH^0, ΔS^0 and ΔG^0 for formation of the PNA-RNA, DNA-RNA, PNA-DNA and DNA-DNA duplexes (Table 1). It is remarkable that the decrease in entropy is almost identical for the formation of DNA-DNA and PNA-DNA duplexes. This implies that the single-stranded PNA has the same degree of organization as single-stranded DNA.

The kinetics of PNA-RNA duplex formation were also measured, and the results show that the rate of hybridization ($\sim 10^6\ M^{-1}\ s^{-1}$, data not shown) is at least as fast as that of DNA-DNA duplex formation. Again, this is fully consistent with the suggestion that the single-stranded PNA is at least as prestructured for duplex formation as is DNA[12].

The molecular forces that are responsible for the unique chemical and biological properties of DNA are still not fully understood. In particular, it is not clear to what extent the Watson–Crick base-pair recognition is an intrinsic property of the nucleobases themselves or how much is due to the rigid structural framework provided by the deoxyribose phosphate backbone. For instance, homo-DNA having hexoses in the backbone does not display Watson–Crick base pairing[4]. Our finding that an acyclic backbone, such as that of PNA, confers DNA-mimicking hybridization properties argues for a key role of nucleobase stacking. Of course we cannot rule out that fortuitous intramolecular interactions of the backbone direct this DNA-like geometry. But it is unlikely that such interactions in PNA-DNA hybrids would be favourable regardless of the orientation of the PNA relative to the DNA; stable PNA-DNA hybrids are formed in both the parallel and antiparallel orientation (Table 1).

TABLE 1 Melting temperatures T_m (°C) for PNA-DNA, PNA-RNA, DNA-DNA and DNA-RNA complexes

First-strand sequence*	TGTACGTCACAACTA†	GTAGATCACT‡	AGTGATCTAC‡
PNA:DNA (parallel)	56.1	38.0	38.0
PNA:DNA (antiparallel)	69.5	51.0	49.0
PNA:RNA (parallel)	51.2	ND	ND
PNA:RNA (antiparallel)	72.3	ND	ND
DNA:DNA	53.3	33.5	33.5
DNA:RNA	50.6	ND	ND

Absorbance versus temperature curves were measured at 260 nm in 100 mM NaCl, 10 mM sodium phosphate, 0.1 mM EDTA, pH 7, as described in ref. 11. T_m, the temperature at which half of the molecules are hybridized, was obtained by fitting triplicate melting curves at 4 μM of each strand to a modified two-state model with linear sloping baseline[20].

* Written 5′–3′ for oligonucleotides and N to C terminal for PNA.
† The PNA terminates in a carboxamide.
‡ The PNA terminates in a lysine amide.

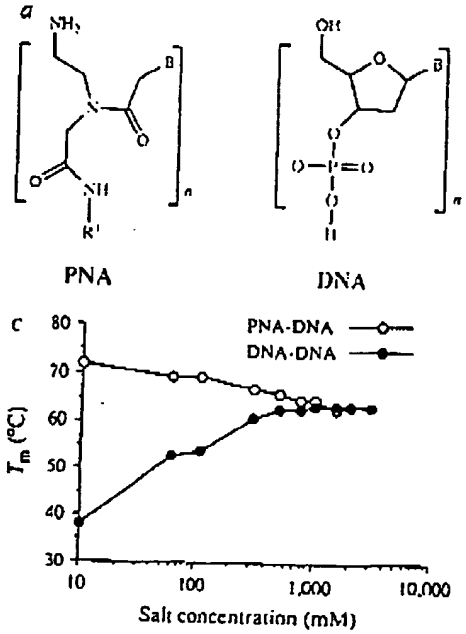
The PNA backbone is uncharged, and we ascribe the increased thermal stability of the PNA-DNA duplex relative to that of the DNA-DNA duplex predominantly to lack of electrostatic repulsion between the two strands. This contention is supported by experiments showing that PNA-DNA and DNA-DNA duplexes have equal thermal stability at ionic strength above 1 M Na^+ (Fig. 1*c*).

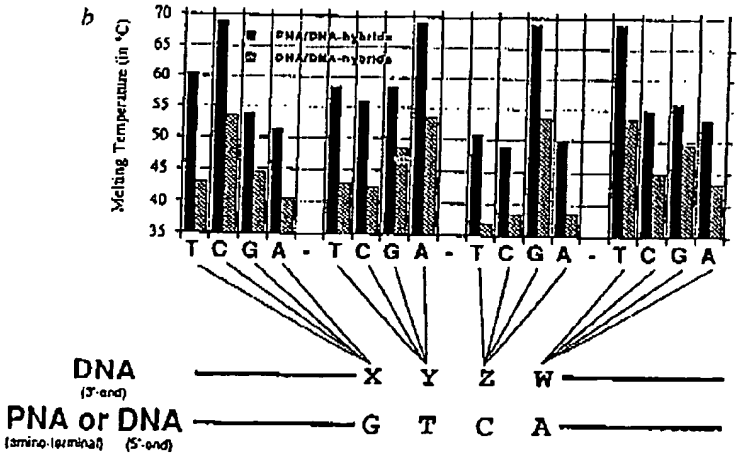
These results may also throw light on the evolution of genetic material. It is now commonly argued that our present-day DNA

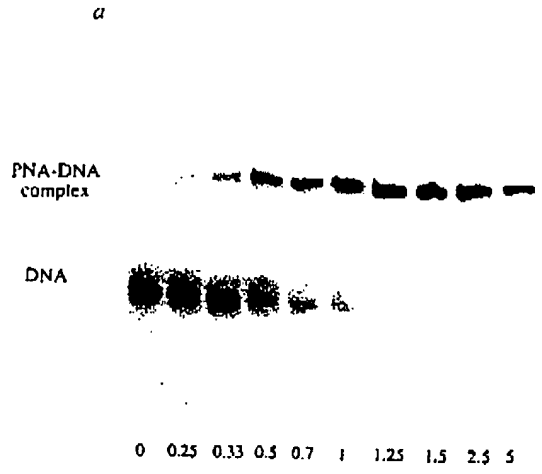
TABLE 2 Thermodynamic parameters for the formation of PNA-DNA, PNA-RNA, DNA-RNA and DNA-DNA duplexes with the sequence TGTACGTCACAACTA present in the PNA strand strand

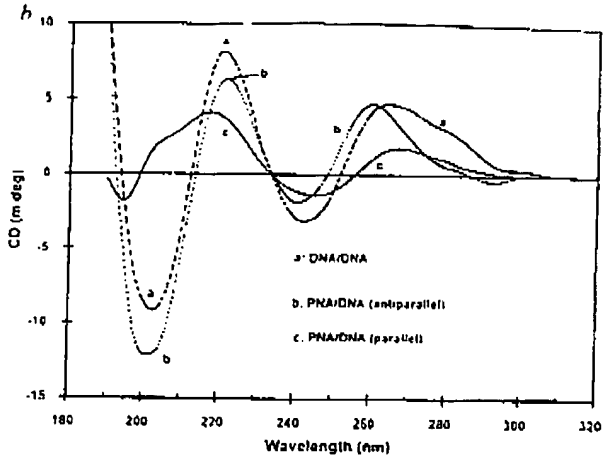
	DNA:RNA	PNA:RNA	DNA:DNA	PNA:DNA
ΔH° (kcal mol^{-1})*	−128.9	−128.5	−105.3	−106.6
ΔS° (EU)*	−372.8	−345.9	−296.2	−285.8
ΔG°_{37} (kcal mol^{-1})*	−13.3	−21.2	−13.4	−18.0
T_m (°C, 8 μM)*	50.1	72.2	53.5	68.8

Measured in 100 mM NaCl, 10 mM sodium phosphate, 0.1 mM EDTA, pH 7.0.
* Obtained from linear plots of $1/T_m$ versus log(concentration)[20].









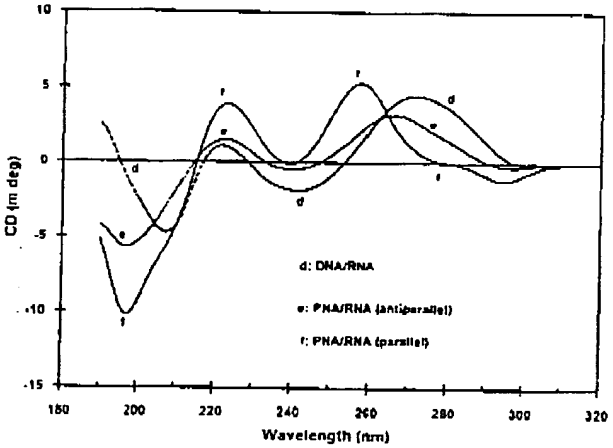


FIG. 2 Structural properties of PNA-DNA complexes. *a*, Titration by gel-shift of the binding of PNA H-TGTACGTCACAACTA-NH_2 to the 5'-end labelled oligonucleotide 3'-d(ACATGCAGTGTTGAT). *b*, Circular dichroism spectra of PNA-DNA (b, antiparallel; c, parallel), PNA-RNA (e, antiparallel; f, parallel), DNA-DNA (a) and DNA-RNA (d) complexes. The DNA sequence (a–c) was 3'-d(ACATGCAGTGTTGAT), and the RNA sequence (d–f) was 3'-ACAUGCAGUGUUGAU.

METHODS. The oligonucleotide was labelled with ^{32}P at the 5' end using standard techniques[21]. The oligonucleotide (1 nmol; 10^3 c.p.m.) was incubated with various amounts of PNA (0–5 nmol) in 10 µl 10 mM Tris-HCl, 1 mM EDTA, pH 7.4, for 1 h at 37 °C. The samples were analysed by electrophoresis in 20% polyacrylamide gels (TBE buffer, 89 mM Tris-borate, pH 8.3, 1 mM EDTA) and the radiolabelled DNA visualized by autoradiography. Concentrations of oligonucleotides and PNA were measured photometrically. Similar results were obtained using the complementary oligonucleotide of reversed polarity (5'-d(ACATGCAGTGTTGAT)). Complexes for circular dichroism were formed by mixing equal molar amounts of the two complements in distilled H_2O. Circular dichroism spectra were recorded on a Jasco 700 instrument at room temperature using an optical path of 1 mm. All measurements were averaged 10 times and smoothed.

world was preceded by an RNA world[13,14]. But RNA is a chemically fragile molecule, unlikely to survive the harsh prebiotic conditions. Note that a 'peptide nucleic acid' like PNA has the recognition properties of DNA and consequently the potential to carry genetic information. It has been shown that both amino acids[15] and nucleobases[16] are formed under conditions designed to mimic the 'prebiotic soup'. Thus it is conceivable that 'peptide nucleic acid'-like compounds could also have been formed, and might have played a prebiotic role. Finally we note that the properties of PNA reported here, especially their capacity for sequence recognition and hybrid stability, emphasize the potential of such compounds as antisense drugs[17,18]. □

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Peptide nucleic acid (PNA). A DNA mimic with a pseudopeptide backbone

CHEMICAL SOCIETY REVIEWS

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PNA (peptide nucleic acid) is a DNA mimic with a pseudopeptide backbone composed of *N*-(2-aminoethyl)glycine units with the nucleobases attached to the glycine nitrogen *via* carbonyl methylene linkers. PNA was first described in 1991 and has since then attracted broad attention within the fields of bioorganic chemistry, medicinal chemistry, physical chemistry and molecular biology due to its chemical and physical properties, in particular with regard to efficient and sequence specific binding to both single stranded RNA and DNA as well as to double stranded DNA. The present review discusses the structural features that provide the DNA mimicking properties of PNA and gives an overview of structural backbone modifications of PNA.

1 Introduction

Self-recognition by nucleic acids is one of the fundamental processes of life and also one of the most straightforward principles of molecular recognition in complex systems. Only four basal recognition units exist, the nucleobases adenine (A), cytosine (C), guanine (G) and thymine (T) [uracil (U) in RNA], that recognize each other two by two forming A–T and G–C base pairs by simple hydrogen bonding between complementary hydrogen bonding acceptor and donor sites of the nucleobases (Fig. 1). Thus, it is not surprising that this simple four building block system has been a paradigm and inspiration for chemists attempting to and succeeding in making self-organizing systems (*e.g.* ref. 1). Also, a large number of close as well as more distant analogues of DNA itself by modifications of the sugar phosphodiester backbone have also been made mainly with the aim of developing efficient antisense drugs

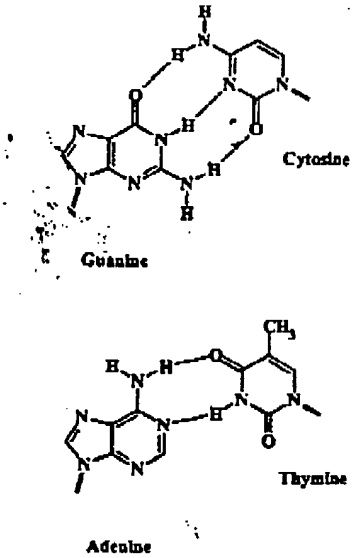

Fig. 1 Nucleobase pair recognition by Watson–Crick hydrogen bonding

(Fig. 2).[2] Peptide nucleic acid (PNA) represents a much more dramatic deviation from the natural DNA structure since the entire phosphodiester backbone has been replaced by a pseudopeptide backbone (Fig. 3). Thus, from a chemical point of view PNA is a hybrid between an oligonucleotide (the nucleobases) and a 'protein' (the backbone) and consequently shows properties from both 'worlds'.

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Peter E. Nielsen

Gerald Haaima hails from the 'Pride of the South', Otago, New Zealand. He obtained his BSc with first class honours in 1985 and followed this up with a PhD (1988) under the supervision of Rex Weavers. After a year with Gary Molander (Boulder, USA) and two years with Lew N. Mander (ANU, Australia) he relocated to Denmark and started work with the late Ole Buchardt and Peter Nielsen on Peptide Nucleic Acids. His research interests include nucleic acid recognition and the development of novel molecular recognition systems.

Gerald Haaima

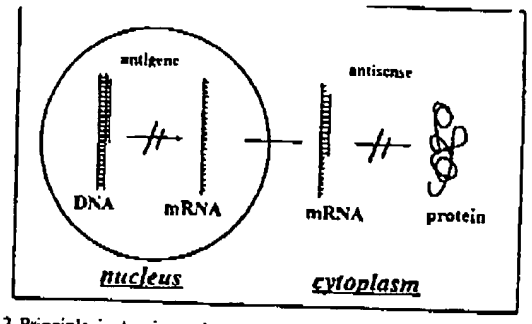


Fig. 2 Principle in 'antisense' and 'antigene' strategies. In the antisense strategy, an oligonucleotide (analogue) binds to the mRNA by Watson–Crick hybridization and thereby inhibits the translation to the protein product. This can occur either by inducing RNAse H degradation of the mRNA or by physical blocking of the ribosomes. An antigene agent binds to the gene itself, the double stranded DNA either by triplex formation (oligonucleotides) or duplex invasion (PNA) and thereby inhibits the transcription of the gene to mRNA.

PNA was originally designed as a reagent to sequence specifically target double stranded DNA as a mimic of triplex forming oligonucleotides which bind as a third strand in the major groove of a DNA double helix *via* T·A–T and C$^+$·G–C Hoogsteen base pairing (Fig. 4).[3] However, the PNA backbone turned out to be a much better substitute for the normal sugar phosphate backbone than anticipated,[4] and therefore much effort has been devoted to exploring the general DNA mimicking properties of PNA as well as its potential as an antisense and antigene drug, including being a sequence specific ligand for binding to double stranded DNA.

The chemistry,[5] physical chemistry[6] as well as molecular biology/drug aspects of PNA[7] have been presented quite extensively in the recent reviews. The present paper will focus on the 'structure–activity' relationships of peptide-like backbones in terms of DNA mimics.

Briefly, PNA is composed of a backbone built up from aminoethylglycine units (a reduced dipeptide backbone) in which the nucleobase is attached to the central amine *via* an

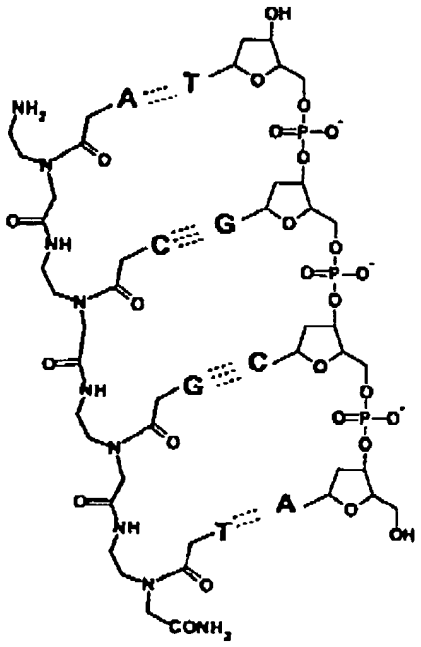


Fig. 3 Chemical structures of DNA and PNA. A, C, G and T designate the nucleobases adenine, cytosine, guanine or thymine.

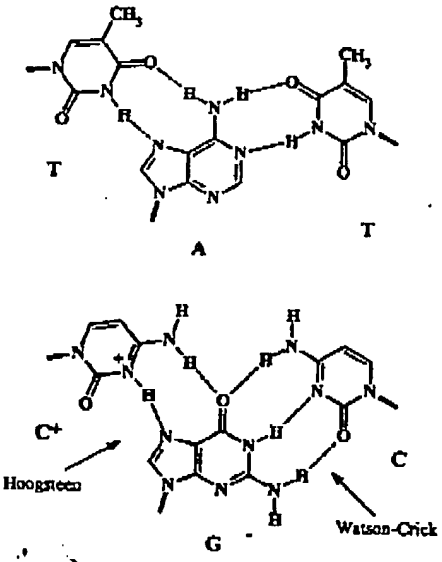


Fig. 4 T·A–T and C$^+$·G–C triplets involving Hoogsteen and Watson–Crick base pairings. Note, that N^3 of cytosine needs to be protonated in order to donate a hydrogen-bond to the N^7 of guanine.

acetyl linker. This particular arrangement of atoms resembles the '6 + 3' number of bonds arrangement found for DNA. A simple geometry dissection of the DNA backbone reveals that six bonds separate each nucleobase unit, while the distance between the backbone and the nucleobase is three bonds. There have been a number of other approaches to the synthesis of PNA monomers[8–10] since the first reports on PNA,[11–13] all of which essentially disconnect the molecule about the central amide bond presenting a synthesis of suitably protected nucleobase acetic acid and a protected backbone. The chemistry for these is well established in the literature and does not present new synthetic problems. More *de novo* approaches have been reported in which the monomer is built up from simple units during the solid phase oligomerization. This approach removes the need for monomer synthesis but its utility in producing high quality product has not been demonstrated.[10] As with peptide synthesis, PNA monomers come in two major varieties, Boc and Fmoc each of which present their own possibilities and limitations. This has been reviewed recently,[5] and by way of example a set of Boc-monomers are shown in Fig. 5.

2 PNA hybridization

PNA oligomers bind strongly and with high sequence discrimination to complementary oligomers of DNA, RNA or another PNA, and in general the hybrid thermal stabilities (T_m) for identical sequences follow the order: PNA–PNA > PNA–RNA > PNA–DNA (> RNA–DNA > DNA–DNA).[4,14] Furthermore, the stabilities of PNA hybrids are, in contrast to hybrids between two anionic oligomers like DNA–DNA or RNA–DNA, fairly independent of ionic strength because of the neutral PNA backbone.[15] It is also noteworthy that PNA hybrids can be formed both in the antiparallel (amino-terminal of PNA facing the 3′-end of the oligonucleotide) and the parallel configuration even though the antiparallel complexes have the higher stability (in general a ΔT_m of 1–2 °C per base pair between antiparallel and parallel complexes are observed).[4]

Homopyrimidine PNAs distinguish themselves by forming PNA–NA–PNA triplexes of extremely high thermal stability.[4,16] Again, the charge neutral backbone of PNA can account for at least part of the triplex stabilization, but an X-ray crystal structure of a PNA$_2$–DNA triplex shows specific interactions (hydrogen bonding) between each amide N–H of the backbone

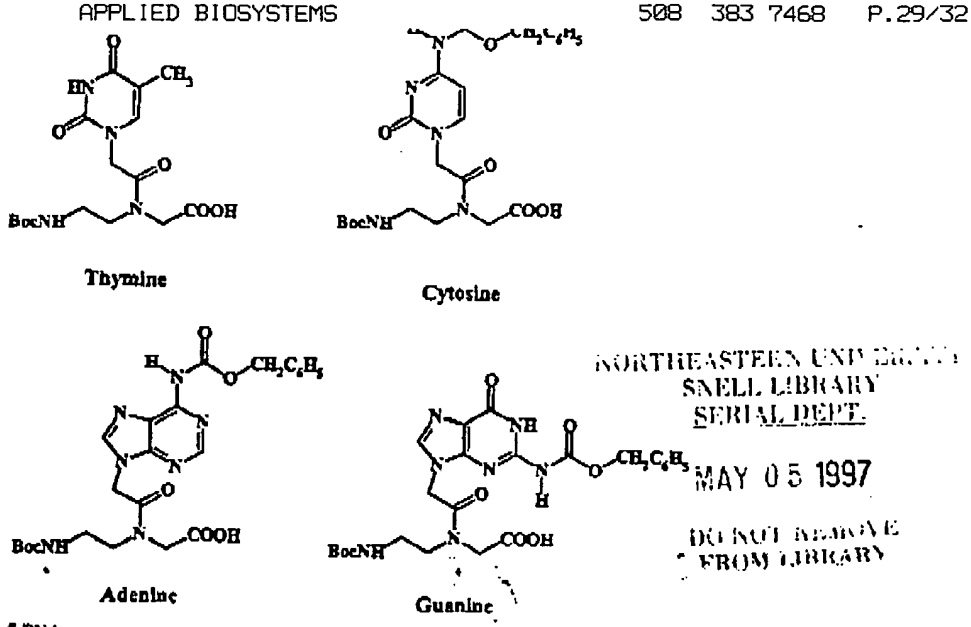


Fig. 5 PNA monomers used for oligomerization using the Boc (*N*-*t*-butoxycarbonyl) strategy

of the Hoogsteen PNA strand and a phosphate oxygen of the DNA backbone[17] thereby also contributing to the stability.

The high stability of PNA_2–DNA triplexes also helps explain why hompyrimidine PNAs when targeted to double stranded DNA prefer not to form traditional PNA–DNA_2 triplexes, but instead invade the DNA double helix forming an internal PNA_2–DNA triplex (having conventional Hoogsteen and Watson–Crick nucleobase interactions) in a strand displacement complex[18,19] (Fig. 6). This novel binding mode has opened a new avenue for the attempts to develop sequence specific dsDNA binding ligands, *e.g.* as gene therapeutic agents (antigene strategy)[20,21] or as biomolecular tools in genome analyses.[22]

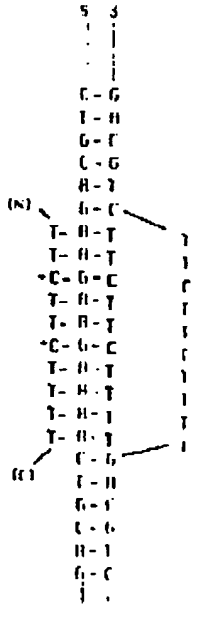


Fig. 6 Schematic representation of a PNA–triplex strand displacement complex involving a PNA–DNA–PNA triplex *via* Watson–Crick and Hoogsteen hydrogen bonding (PNA is shown in heavier type than DNA)

3 Biological aspects of PNA

PNA has many of the properties of a promising antisense or antigene drug, such as stable and highly sequence specific binding to the complementary mRNA or dsDNA gene target, high biological and chemical stability.[23] The easy synthetic accessibility and not least synthetic flexibility of PNA should also allow further optimization of the structure, especially with regard to bioavailability and pharmacokinetic properties. Thus it is not surprising that the drug aspects together with the utility as a molecular biology tool of PNA technology is being actively pursued, and the results so far are very encouraging.[7]

4 PNA structure

Four high resolution structures of PNA complexes are available at present. Two structures, a PNA–RNA[24] and a PNA–DNA[25] duplex, were determined by NMR methods, while a PNA_2–DNA triplex[17] and a PNA–PNA duplex[26] were solved by X-ray crystallography. It is quite clear from these structures that the PNA oligomer is to some extent able to structurally adapt to the oligonucleotide complement, but it is equally clear that the PNA has a preferred structure of its own termed the 'P-form'.[17,26] This is, of course, most obvious from the structure of the pure PNA duplex which is a very wide (28 Å diameter) helix with an accordingly large base pair helical displacement and a very large pitch (18 bp) [Fig. 7(*a*)]. A canonical B-form helix which is typical for DNA duplexes has a diameter of *ca.* 20 Å and a pitch of ten base pairs per turn. The base pairs are perpendicular to the helix axis and stack through the centre of the helix. A canonical A-form helix, typical of RNA duplexes, also has a diameter of *ca.* 20 Å but a pitch of 11 base pairs per turn, and the base pairs are tilted *ca.* 20° relative to the helix axis. Furthermore, the base pairs are displaced away from the helix leaving a central 'tunnel' in the helix, analogous to that seen in the P-form. It is also noteworthy that the base pairs in the P-form are practically perpendicular to the helix axis (B-like) with only minor variations in slide, tilt and propeller twist angles between individual base pairs, and with an interbase-pair stacking overlap that is remarkably close to that found in canonical A-form RNA helices (Fig. 7). It is likewise notable that the

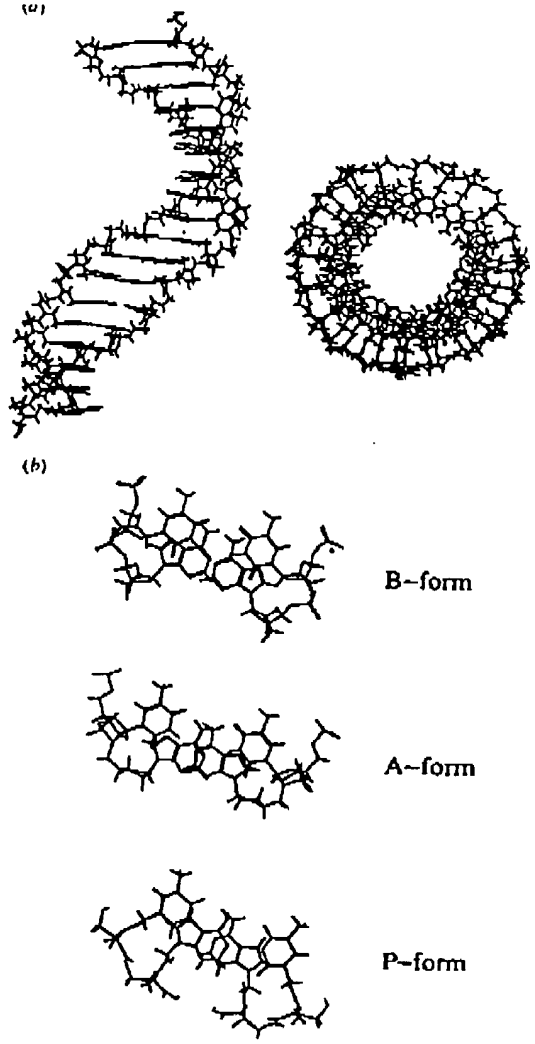


Fig. 7 (*a*) Structure of a PNA duplex from X-ray crystallography. The structure was determined from a self-complementary hexamer[26] but the full turn (18 bp) of the helix has been modelled from these data. Only the right-handed form is shown, but the unit cell contains both a right-handed and a left-handed form. (*b*) Base pair overlaps in A-, B- and P-form helices. Two consecutive A–T base pairs are shown as viewed from the end of the helix for a canonical B-form, a canonical A-form or for the crystal structure of the PNA–PNA duplex P-form.

backbone structure found in the PNA duplex[26] is almost identical to that seen in both the Watson–Crick and the Hoogsteen strand of the PNA_2–DNA triplex[17] and that the basic features, such as carbonyl orientations are also in common with the two PNA–oligonucleotide duplexes.[6,24,25]

One conclusion to be drawn from the above described structural data is that despite the ability of PNA to efficiently bind and recognize DNA or RNA, the conformation adopted by PNA in these hybrid complexes is not optimal, because the P-form helix preferred by PNA is distinct in terms of important parameters such as helical width and pitch from both the B-form preferred by DNA and the A-form of RNA. Thus, one should be able to obtain a better peptide nucleic acid DNA mimic if one could construct a backbone that in its lowest energy state would adopt a B-form (or A-form) helix. However, the compactness and simplicity of the PNA structure pose severe restrictions as to which modification can be implemented and still result in chemically reasonable structures. Some of these possibilities are discussed below.

5 PNA backbone modifications

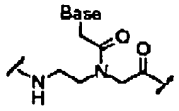
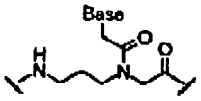
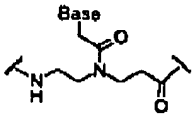
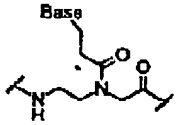
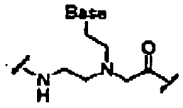
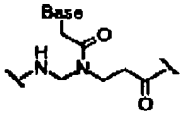
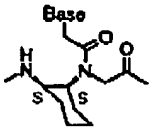
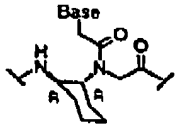
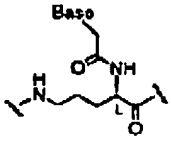
Since the first reports on PNA, a large number of PNA backbone derivatives have been described and investigated (Tables 1 and 2) in order to explore the 'structural space' in which this type of PNA mimic operates, as well as in an effort to obtain a molecular understanding of the chemical and structural parameters that determine the DNA mimicking properties of a (peptide) nucleic acid analogue. Thereby we should hopefully also gain a better understanding of the DNA (and RNA) molecules themselves.

The results so far have shown (Tables 1 and 2) that only certain alterations of—or deviations from—the original aminoethyl-glycine backbone are 'allowed' without severe penalties in DNA/RNA–PNA hybrid stability. As an overall conclusion at this stage one cannot touch the basic structure of the PNA backbone (1), *e.g.* by extending either of the 'linkers' [ethyl → propyl (2), glycine → β-alanine (3) or acetyl → propionyl (4)],[27] reducing the nucleobase linker amide (5)[28] or even reverse the amide linkage within the backbone (6).[29] However, much freedom seems to be in placing (functional) substituents on the backbone as exemplified by exchanging the non-functional glycine for other natural amino acids[30] (**10–18**, Table 2), although the type of substituent and also the stereochemistry at the now created chiral centre have different effects on the PNA hybridization properties. Even cyclic substituents, as exemplified by the 'cyclohexyl substitution' at the amino ethyl linker (**8**, **9**) is possible provided the 'right' stereoisomer is chosen (Lagriffoule, Nielsen *et al.*, in prep.).

Naturally, the PNA analogues described to date have far from exhausted the imagination of chemists and more will undoubtedly be investigated, now that 'pure peptide chemistry' has been introduced successfully in the 'oligonucleotide analogue' field. More bold chemists may even do away with both sugar–phosphate and peptide backbones and come up with totally novel constructions.

6 Why is PNA a good DNA mimic?

One might ask: what features of the original PNA structure are responsible and required for its DNA mimicking properties and also what improvements might be possible? However, prior to engaging in this discussion, it is informative to make some thermodynamic considerations. Hybridization of complementary oligomers whether these being DNA, RNA or RNA is characterized by a large enthalpy gain and a significant entropy loss.[4,15] The decrease in entropy upon hybrid formation, naturally, is due to the formation of a highly ordered and fairly rigid duplex structure from two rather flexible and much less ordered single strands. (It should be kept in mind that an entropy gain which cannot compensate for the above loss is also accompanying hybrid formation due to release of ordered water molecules around the hydrophobic nucleobases). Therefore constraining the single stranded PNA (or other oligomer) in a conformation identical to or close to that found in the hybrid should greatly reduce the entropy loss and therefore increase the free energy upon binding. Thus restricting backbone flexibility, *e.g.* by introducing cyclic structures is an obvious strategy in the quest for oligomers of improved hybridization potency. This principle has been met with some success using bicyclic DNA analogues for triplex formation,[31] and the idea was also the rationale for making the cyclohexyl derivatives **7** and **8** of PNA (Lagriffoule, Nielsen *et al.*) (Table 1). Disappointingly, neither the (*SS*)- nor the (*RR*)-isomer conferred improved hybridization although the (*SS*)-isomer had no serious adverse effect. Very interestingly, however, a thermodynamic analysis revealed that when compared to a normal PNA, a 10-mer PNA containing three (*SS*)-cyclohexyl units only showed an entropy loss ($-\Delta S$) of 280 J mol^{-1} K^{-1} (*versus* 375 J mol^{-1} K^{-1} for the normal PNA), whereas the enthalpy gain ($-\Delta H$) was 127 kJ mol^{-1} (*versus* 153 kJ mol^{-1}). Therefore, one may conclude that the

Entry	Structure	Backbone/linker	ΔT_m DNA/ °C	ΔT_m RNA/ °C	Ref.
1		Ethylglycine	0	0	
2		Propylglycine	−8.0	−6.5	27
3		Ethyl-β-alanine	−10	−7.5	27
4		Propionyl linker	−20	−16	27
5		Ethyl linker	−22	−18	28
6		Retro-inverso	−6.5	nd	29
7		(*S,S*)-Cyclohexyl	−0.7	−0.5	Submitted
8		(*R,R*)-Cyclohexyl	−8	−7	Submited
9		L-Ornithine	−14	−8	34, 35

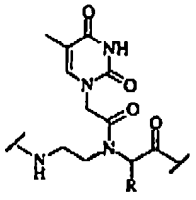
structural constraints most likely has had the desired effect of producing a more ordered single strand state, but, unfortunately, not in the optimal conformation(s) for DNA (or RNA) hybridization.

Although thermodynamic data are not yet available for other PNA derivatives, it is obvious that in addition to any adverse steric or structural constraints imposed by the changes of derivatives **1**–**5**, these will all result in more flexible molecules. This is especially illustrative for derivative **5**, in which all distances are retained, but one of the amide bonds has been reduced.

Therefore apart from having the proper 'intra-backbone' distances (6 + 3), we believe that the constrained flexibility imposed by the two amide functions in the PNA backbone is crucial. However, the poor DNA mimicking properties of the 'retro-inverso' PNA (**6**) which is a true isomer of the original aminoethylglycine PNA obeying both the '6 + 3-rule' as well as having the same number of constraining amide bonds, shows that more subtle factors such as dipole–dipole interactions and changes in hydration patterns that we do not fully understand also make significant contributions. On the other hand, using the 'PNA system'—due to the synthetic accessibility of a wide

variety of analogues—could help us further unravel the general principles for structure–activity relationships at the molecular level as well as improve our ability to translate chemical structures into three-dimensional structures.

Table 2 Effects on thermal stability per monomer (ΔT_m/°C) for the PNA sequence H–GTA GAT CAC T–NH_2[a] incorporating three chiral monomers as compared to an unmodified PNA[30]



Entry	R	Chirality	ΔT_m DNA/°C	ΔT_m RNA/°C
10	CH_3	L	−1.8	nd
11	CH_3	D	−0.7	nd
12	Bu[t]	L	−2.6	−3.0
13	CH_2OH	L	−1.0	−1.0
14	CH_2OH	D	−0.6	−1.0
15	CH_2CO_2H	L	−3.3	nd
16	$CH_2CH_2CO_2H$	D	−2.3	nd
17	$(CH_2)_4NH_2$	L	−1.0	−1.3
18	$(CH_2)_4NH_2$	D	+1.0	0

7 Outlook

The results obtained with PNA have bearing on many areas of chemistry and biology ranging from basal molecular recognition, self-assembly and chiral induction aspects[14] over molecular biology tools and gene therapeutic drugs to our understanding of the structure and function of Nature's genetic material, DNA, and its possible prebiotic predecessors and origin.[32] Even some novel materials have their origin in PNA.[33] Therefore, PNA should not be viewed only as a DNA mimic, but as a structural and self-recognizing system in its own right, and we foresee that the properties of PNA and related compounds will prove of increasing interest and utility in both the traditional 'oligonucleotide field' as well as in other areas of science—including ones which at this stage are not imagined.

8 Acknowledgements

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